

LEE et al.
Appl. No. 09/786,521
August 6, 2003

- CLAIMS PTO -
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ECW

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. Canceled.
2. Canceled.
- 3.-16. Canceled.

17. (new) A method of monitoring the temperature of a biochemical reaction in a reaction mixture, said method comprising effecting the reaction in the presence of a fluorescently labelled temperature probe DNA sequence which comprises a double stranded region which denatures at a predetermined temperature, the fluorescent label of said temperature probe sequence being arranged so that a detectable signal occurs at the point at which denaturation of said region takes place; and monitoring fluorescence from said reaction mixture so as to determine when said predetermined temperature has been reached, wherein the temperature probe comprises a single nucleic acid strand, end regions of which hybridise together so as to form a loop or hairpin structure and wherein the fluorescent label comprises an intercalating dye.

2 18. (new) A method of monitoring the temperature of a biochemical reaction in a reaction mixture, said method comprising effecting the reaction in the presence of a fluorescently labelled temperature probe DNA sequence which comprises a double stranded region which denatures at a predetermined temperature, the fluorescent label of said temperature probe sequence being arranged so that a detectable signal occurs at the

point at which denaturation of said region takes place; and monitoring fluorescence from said reaction mixture so as to determine when said predetermined temperature has been reached, wherein the fluorescent label utilises fluorescence transfer (FRET) as the basis of the signal and wherein the temperature probe DNA sequence is provided with a reporter and a quencher molecule, the reporter and quencher molecules being located on different strands of a DNA temperature probe sequence, and arranged such that on hybridisation of the strands, they are brought into close proximity to each other.

3 ~~19.~~ ² (new) A method according to Claim ~~18~~ wherein FRET is established between an intercalating dye and a quencher molecule arranged on a strand of the temperature probe sequence such that it can absorb radiation from dye which is in close proximity on hybridisation of the strands.

4 ~~20.~~ (new) A method of monitoring the temperature of a biochemical reaction in a reaction mixture, said method comprising effecting the reaction in the presence of a fluorescently labelled temperature probe DNA sequence which comprises a double stranded region which denatures at a predetermined temperature, the fluorescent label of said temperature probe sequence being arranged so that a detectable signal occurs at the point at which denaturation of said region takes place; and monitoring fluorescence from said reaction mixture so as to determine when said predetermined temperature has been reached, wherein the fluorescent label utilises fluorescence transfer (FRET) as the basis of the signal and wherein the temperature probe DNA sequence comprises a first DNA strand having a reporter molecule thereon, a second DNA strand having a quencher

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molecule thereon, said first and second DNA strands being designed to hybridise to a third DNA strand such that the reporter and quencher molecules are brought into close proximity with each other.

5 ~~21~~. (new) A method according to Claim ~~17~~¹, ~~18~~², ~~19~~³ or ~~20~~⁴ wherein the biochemical reaction is an amplification reaction.

6 ~~22~~. (new) A method according to Claim ~~21~~⁵ wherein the amplification reaction is a polymerase chain reaction.

7 ~~23~~. (new) A method according to Claim ~~22~~⁶ wherein the length of the temperature probe is similar to that of an amplicon of the polymerase chain reaction.